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RESEARCH ARTICLE

Effect of Strenuous Physical Activity on Cortisol and Leukocyte in Mice

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ABSTRAK

Pendahuluan: Aktivitas fisik berat selain menyebabkan peningkatan radikal bebas juga dapat menyebabkan stres fisik, psikis, dan penurunan imunitas. Stres psikis terbukti mempengaruhi poros hipothalamus, hipofisis, dan cortek adrenal sehinga mampu meningkatkan kadar cortisol dan apoptosis sel darah. Namun pengaruh aktivitas fisik berat terhadap peningkatan kadar kortisol dan jumlah leukosit masih inkonsisten. **Tujuan:** Untuk mengetahui pengaruh exercise berat terhadap peningkatan kadar hormone kortisol dan penurunan jumlah leukosit.

Metode: Dalam *Post Test Only Control Group Design*, sebanyak 15 ekor mencit jantan BALB/c, dikelompokkan menjadi 3 kelompok secara random. Kelompok kontrol (G-0) tidak dilakukan aktifitas fisik, kelompok perlakuan aktivitas fisik berat 1x (G-1), dan (G-2) kelompok aktifitas fisik berat 3 kali. Semua kelompok dilakukan pemeriksaan kadar kortisol dengan metode ELISA dan pemeriksaan jumlah leukosit dengan metode Direct counting. Analisis data dengan uji *One Way ANOVA* dilanjutkan dengan *Post Hoc* dan *Korelasi Pearson,* dengan tingkat kepercayaan 95%.

Hasil: Rerata kadar kortisol pada G-0 11,86±2,10ug/dl, G-1, 32,00±0,86ug/dl,danG-2, 44,58±1,74ug/dl. Jumlah leukosit pada G-0, 6180±540,37/mm3, G-1, 4650±217,94/mm3, dan G-2, 4180±130,38/mm3. *Uji One Way Anova* masing-masing menunjukkan perbedaan secara bermakna di antara kelompok, $p < 0.001$. *Post Hoc Test* menunjukkan bahwa kadar kortisol pada G-1 dan G-2 lebih tinggi secara bermakna dibanding G-0, $p < 0.001$. Sedangkan jumlah leukosit pada G-1 dan G-2 lebih rendah dibanding G-0, $p < 0.001$. Terjadi korelasi negatif antara kadar *cortisol* dan jumlah leukosit ($r = -20,14$) secara signifikan, $p < 0,001$.

Kesimpulan: Aktivitas fisikberat terbukti mampu meningkatkan kadar kortisol dan penurunan jumlah leukosit pada mencit BALB/c.

Kata kunci: aktivitas fisik berat, kadar kortisol, jumlah leukosit.

ABSTRACT

Introduction: *Strenuous physical activities cause an increase in free radicals, physical stress, psychological stress and decreased immunity. Psychological stress affects the hypothalamic-pituitary-adrenal (HPA) axis leading to increased levels of cortisol and blood cell apoptosis. However, the studies on the effect of strenuous physical activities on cortisol levels and leukocyte count showed inconsistent findings.* ***Objectives:*** *To examine the effect of strenuous exercise on cortisol levels and leukocytes count.*

Methods: *In this study with post test only control group design, 15 male BALB/c mice were randomly assinged to the following 3 groups: control (non-exercised) group (G-0), once strenouse physical activity (G-1), 3 times strenuous physical activity (G-2). Cortisol levels were evaluated by ELISA.White blood count was determined. Data were analyzed by one way ANOVA test followed by Post Hoc and Pearson, with a confidence level of 95%.*

Results: *The mean cortisol level in group G-0, G-1, G-2 were 11.86 ± 2.10µg/dL, 32.00 ± 0.86µg/dL, 44.58 ± 1.74µg/dL respectively. The number of leukocytes in G-0, G-1, G-2 were 6180±540.37/mm3, 4650±217.94/mm3, 4180±130.38/mm3 respectively. One way ANOVA showed a significant difference between groups ($p < 0.001$). Post Hoc Test showed that the levels cortisol in G-1 and G-2 were significantly higher than thatof G-0 ($p < 0.001$). The number of leukocyte in G-1 and G-2 was lower than that of G-0 ($p < 0.001$). There was a significant negative correlation between cortisol levels and the number of leukocytes ($r = -20.14$, $p < 0.001$).*

Conclusions: *Strenuous physical activity increasescortisol levels and decrease the number of leukocytes in BALB /c mice.*

Keywords: *strenuous physical activity, cortisollevel, the number of leukocytes.*

INTRODUCTION

Regular and voluntary physical activities with a mild to moderate intensity can improve health and fitness. Such aerobic activities can improve and slow down the decline in organ function and the body's resistance against infection. On the other hand, a study reported by Sharkey showed that strenuous physical activityto exhaustion can cause immune disorders

characterized by influenza infection right after the activities (Sharkey, 2011). In addition, physical activity has beenshown to cause severe physical and psychological stress. Psychological stress can physiologically affect the hypothalamus–pituitary–adrenal cortex axis, thus increase cortisol levels. However, studies on the physical effect of strenuous activityon the levels of cortisol and leukocyte count show inconsistent findings.

People avoidingphysical activities and prefering a sedentary life style are likely to be obese. Studies showed that 43.75% of the 80 students of SMAN 9 Semarang had an 8-hour sleep, more than 3 hour watching televisionin a day (Wiwied, 2012). Hanley *et al* conducting research among Canadian found that adolescents aged 10-19 watching televisionmore than 5 hours per day were significantly more likely to experience nutritional excess (Hanley, 2006). If people with a sedentary lifestyle performa strenuous physical activities, they can have the female athlete triad syndrome (FAT) as experienced by weightlifting athletes in Yogyakarta. The athletes having risks of FAT with a very high, high, moderate, low and very low risk were 3 11.54%, 11.54%, 50.00%, 19.23%, 3.85% respectively. Meanwhile, based on the FAT risk factors, 4 athletes (15.38%) had a very high risk, 5 athletes (19.23%) had a high risk, 12 athletes (46.15%)had a moderate risk, 2 athletes (7.69%) had a low risk, 3 athletes (11.54%) had a very low risk (Rismayanthi, 2010).

Cheng Zheng and Arizumi showed that cold stress in rats resulted in increased levels of cortisol and affect the immune system (Arizumi, 2007). According to a study conducted by Sari, after subjected to the maximum physical activity, the mice had increased leukocyte and lymphocyte counts. The maximum activity also reduced neutrophils, eosinophils, monocytes but not basophils counts (Sari, 2008). Ali Shaukat also showed leukocytosis occurs in un trained subjects subjected to a strenuous physical activities. On the other hand,Gren *et al.,* reported that 4 out of 20 marathon runner had a lowwhite cell count(4.3 x 103 per uL) physical exercise (Shaukat, 2008).

A strenuous physical activity can increase the oxygen uptake by 100-200 folds due to an increase in metabolism. Increased oxygen uptake during physical activity leading to increasedformation of reactive oxygen species (ROS) (Collet, J, 2014). Generally 2-5% of the oxygen used in the metabolic process of oxidative phosphorylation in the mitochondria will be the superoxide ion, a free radical generated during strenuous physical activity (Chevion S, 2011). Free radicals are molecular species with unpaired electrons in the outermost bonding orbital and highly reactive to cells or cell components (Shaum, 2013). Because its reactivity, free radicals can cause damage to cells and cellular components such as lipids, proteins and DNA, and can cause mutations and are carcinogenic (Kumar V, 2008). Body has a mechanism to respond to the impaired body part through the process of homeostasis. The process is influenced by local and systemic factors (Guzel NA 2007). One of the systemic factors is glucocorticoid hormone in whichmost of itsactivities arecarried out by cortisol. Cortisol is produced by the adrenal glands cortex and regulated by the hypothalamus through Hipotalamic-Pituitary-Adrenal (HPA) axis. HPA will activate the stress response including an increase in cortisol, hypermetabolism, aldosterone, and Adrenocorticotropic Hormone (ACTH) (Guzel NA, 2007).Glucocorticoids affect the movement and function of leukocytes leading to leukocytes cell death, suppressing the immune system, causing the decreased production of lymphocytes, eosinophils, monocytes and basophils (Guyton and Hall, 2014). The purpose of this study was to determine the effect of strenouse physical activity on levels of cortisol and leukocyte count, as well as their correlations.

METHODS

This was a laboratory experimental study with post test only control group design (Sastroasmoro S, 2014). The study was conducted in the laboratory of Pharmacy STIKES Kendal. A total of 15 BALB/c mice aged 2-months, weighing 30-35 grams were assigned to the following 3 groups: Untreated mice (G-0), treated with strenouse physical activity such as maximum swimming activities until almost drowned with load of 9% (2.9 grams) of body weight in the tail (G-1), treated with the same form of strenuous physical activity with G-2 once a day for 3 days (G-2). Blood sampling on orbital sinus of the G-0, G-1 was collected immediately after treatment, while the G-2 on day 3 after the last treatment for the examination of cortisol and leukocytes.

The Level of Cortisol

The level of cortisol was evaluated by ELISA, blood samples and anti-rabbit-alkaline phosphatase antibodies were incubated for 30 minutes at a temperature of 37degree C in the test unit. Then enzyme conjugate not bound cleaned by washing sentrifius then added substrate of the test unit and incubated for 10 minutes. Substrate chemilumonescent, phosphate esters of adamantly dioxentane, underwent hydrolysis with alkaline phosphatase forming an unstable intermediate. Continuous production created emissions of light that

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Table 1. Mean Levels of Cortisol and Total leukocytes

Variables	Groups			*P* (ANOVA)
	G-0 N = 5; ± SD	G-1 N =5; ± SD	G-2 N = 5; ± SD	
Cortisol (µg/dl)	11.86 ± 2.10	32.00 ± 0,86	44.58 ± 1.74	0.000
Leukocyte (Σ/mm^3)	6180 ±540.37	4650 ±217.94	41.80 ±130.38	0.000

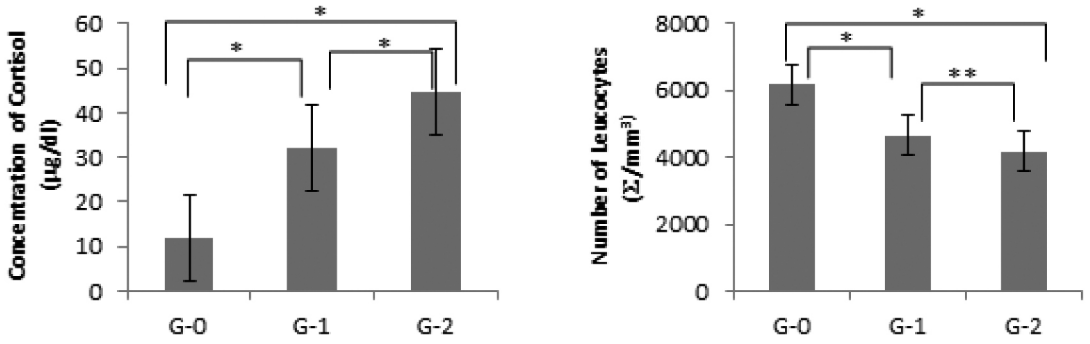

Figure 1. Cortisol levels and number of Leukocyte: *p < 0.001; ** p < 0.005

can be read as cortisol. Cortisol concentrations were measured by luminoler proportional ELISA reader (Baratawidjaja, 2014).

Leukocyte Count

The leukocyte countswere determined by direct counting, the blood was sucked by the WBC diluting pipette up to 0.5 mark, then sucked reagent turk up to 11 mark and shaken several times, and then allowed to stand for 3 minutes. The first three to four drops were removed, then were placed in a counting chamber. leukocytes were examined under a microscope with a weak magnification. Calculations in 4 large boxes, the number of leukocytes/mm3 of blood were determined using the following formula: N x 50 (Gandasoebrata. R, 2006).

Statistic Analysis

The statistical significance was calculated using one-way ANOVA followed by posthoc and Pearson correlation with a 95% confidence interval.

Ethics approval was obtained from FK UNISSULA Semarang prior to the commencement of the study.

RESULTS

The mean time of treatment in the group G-1 was 32.4 minutes, the G-2 31 minutes, 28.6 minutes, and 28 minutes, after examination cortisol levels and the number of leukocytes are shown in Table 1.

The results showed that the highest cortisol levels was found in G-3, followed by the G-2, and the lowest of the G-0, while the lowest number of leukocytes was found in G-3, followed by G-2. The highest number of leukocytes was found in G-0. Due to the normality and homogeneity test each using the Shapiro-Wilk test and Levene test showed normal distribution and homogeneous (p> 0.05), the parametric test requirement was met. The statistical test selected was one way Anova. The results of ANOVA showed that cortisol levels and leukocyte counts were significantly different (p <0.001).

Post hoc analysis results showed that strenuous physical activity can increase cortisol levels of the G-1 significantly compared to the control group (G-0), p <0.001 but lower than in group G-2 (P <0.001). On the other hand, strenuous physical activity in group G-2 significantly reduced the number of leukocytes compared to that of G-0 and G-1 (p <0.001). The number of leukocytes in the G-1 was also lower than that of G-0, p <0.001 and higher compared to that of G2 (p = 0.042) (figure 1).

The correlation between increased levels of cortisol and a decrease in the number of leukocytes after a strenuous physical activity. The results of Pearson correlation analysis showed that there was a correlation between cortisol levels and the number of leukocytes (p <0.001). Pearson r value was negative 0.91 showing the correlation between cortisol levels and the number of leukocytes with a strong negative linear correlation or closely. This illustrates that increased levels of cortisol in the treatment group of a strenuous physical activity

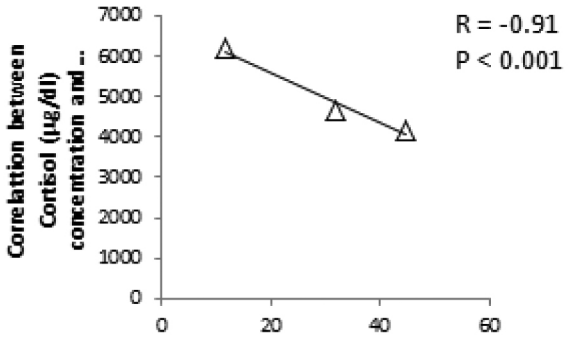

Figure 2. Correlation between Cortisol concentration and Number of Leukocyte

(G-1 and G-2)were followed by a decrease in the number of leukocytes. Meanwhile, the lower cortisol levels were lower in the control group was followed by a high number of leukocytes (figure 2).

DISCUSSION

The results of this study indicate that strenuous physical activities can increase cortisol levels and decrease the number of leukocytes significantly. The findings are consistent with research conducted by Ihalainen *et al.*, in healthy young subjects showing that right after hypertrophic, resistance exercise, the cortisol levels increased significantly (Ihalainen *et al*. 2014). These increased cortisol levels were caused by stress factors, considering that cortisol are hormone generated during stressful conditions. Under normal conditions, cortisol hormones are released in small quantities and gradually throughout the day diurnally thus achieving the highest peak in the morning and lowest in the afternoon or evening (Sarcs, 2012). Cortisol secretion is controlled by corticotropic releasing factor (CRF), secreted by hypothalamus leads to increased levels of Adrenocorticotropin hormone (ACTH) secreted by anterior pituitary activating the adrenal cortex so that the glucocorticoids, especially cortisol secretion is increased (Haksle, 2014; Guyton and Hall, 2006). According toWindu, cortisol levels in male mice in stress conditions will persist for 1 month (Windu, 2014). Meanwhile, Cheng Zheng and Arizumi revealed that cold stress in rats resulted in increased levels of cortisol and affect the immune system (Arizumi, 2007).

On the other hand, a decrease in the number of leukocytes in this study was associated with increased cortisol levels caused by strenuous physical activity. This study showed that there is a strong negative correlation between cortisol levels and the number of leukocytes. On the one hand, the results supports to those of Ihalainen on maximal resistance exercise showing that there is a negative correlation between the acute response to increased levels of cortisol and acute response increase in the number of leukocytes (Ihalainen *et al*. 2014). In contrast, Ihalainen *et al*., the hyperthropic resistance exersice showingthat right after exercise, the number of leukocytes and lymphocytes were significantly increased (Ihalainen *et al*. 2014). This difference, thought to be caused by the different types of metabolism that lasts for menjalanai exersice. In hyperthropic resistance exersice conducted in research Ihalainen type of metabolism that occurs is anaerobic, as evidenced by elevated levels of lactate acid followed by an increase in the number of leukocyte immediately after a hyperthropic resistance exersice. While in this study, with the heavy load on rat while swiming until nearly drown, was an aerobic exercise that type of metabolism that occurs is aerobic followed by increase in lactate. Acid levels in the study were not evaluated lactic acid levels, thus requiring a further research. It is important to note that the study conducted by Ihalainen showed no correlation between elevated cortisol levels and an increase in the number of leukocyte response (Ihalainen *et al*. 2014).

Increased levels of cortisol resulted in a decrease in the number of leukocyte is a non-specific response of the body to stress, thus leading to physiological changes. In long term it results in decreased immune system effectivity (Arizumi 2007). Increased cortisol as the stress response, will inhibit the immune system by decreasing the number of leukocytes circulating in the body, inhibiting leukocyte cell division, decreasing the number of antibody-producing cells and inhibiting the production of antibodies. Cortisol also decreases phagocytosis response and increases apoptosis (programmed cell death) in B cell apoptosis extrinsic pathway is a typical system in the immune system and is used to eliminate activated T cells at the end of the immune response. this pathway is mediated by perforin/Granzyme (Figueiredo, 2008). The reduced number of leukocytes after a strenuous physical activity due to the effect of glucocorticoids on the primary organs producing blood cells undergoing apoptosis, resulting in the formation of cells impaired leukocyte (Sue E, 2012). Apoptosis was not examined in this study, the involvement of extrinsic apoptosis in leukocytes decrease due to increased cortisol levels needs further studies.

CONCLUSION

Strenuous physical activity once in day for 3 days increases cortisol levels and decreases in the number of leukocytes in male BALB/c mice.

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